Biphasic Inhibition of Urokinase-Induced Fibrinolysis by ε-Aminocaproic Acid; Distinction from Tissue Plasminogen Activator* (33661)

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The origin of urokinase (UK), the plasminogen activator prepared in high purity from human urine (1, 2), is still under debate. After its intravenous infusion little or no UK appears in urine (3–5) in spite of a tremendous increase in blood fibrinolytic activity making an excretion from blood doubtful. The kidney and tissues of the urinary excretory tract have been suggested as possible sources (6). Kidney cell cultures produce plasminogen activator (7, 8) and human UK is reported to be immunologically identical with plasminogen activator from human kidney cell cultures though distinct from human plasminogen activator from other sources (9). A plasminogen activator in rabbit kidney lysosome-microsome fractions is reported to resemble human UK (10). Preparations of highly purified tissue plasminogen activator (TA) have been made from pig heart by extraction with dilute acetate at pH 4.2 (11), or from pregnant hog ovaries with molar thiocyanate (12). We want to report some experiments demonstrating striking qualitative and quantitative differences in the behavior of highly purified UK and TA towards a class of inhibitory compounds consisting of ε-aminocaproic acid (EACA), trans-4-aminomethylcyclohexancarboxylic acid (AM CHA), and *p*-aminomethylbenzoic acid (PAMBA), thus distinguishing the two plasminogen activators from each other.

Materials and Methods. Fibrinolytic activity was determined by a lysis time method (13) using solutions of 0.3 ml of bovine fibrinogen (prepared with ammonium sulfate and dialyzed) in saline barbital buffer (SBB) and 0.3 ml of inhibitor (in SBB) clotted with a mixture of 0.1 ml of bovine thrombin and 0.3 ml of activator (both in SBB with gelatin). Final fibrinogen concentration, 0.15%; thrombin concentration, 2 NIH units/ml; ionic strength, 0.15; pH 7.75; reaction temperature, 37°. SBB: 0.05 *M* sodium barbital in 0.1 *M* NaCl, pH 7.75. SBB with 0.25% gelatin was used for activator and thrombin solutions to prevent absorption to glass. UK preparations: I. Leo Pharmaceuticals, 10,000 Ploug units/vial (~ 10,000 CTA units/mg of powder), batch 66021. II. Abbott Laboratories, 50,000 CTA units/mg of protein, batch 2517-110C. III. Sterling-Winthrop Laboratories, 42,400 CTA units/mg of protein, batch R-030-AN. Tissue plasminogen activator: Two preparations, prepared from pregnant hog ovaries by Preben Kok (12), and containing respectively about 10,000 and 150,000 tissue activator units (A and A units) per mg of protein. Concentrations in the final mixtures were adjusted to activities corresponding to 2.5–80 CTA units/ml yielding lysis times from 32 to 6 min in absence of inhibitor. Plotted double logarithmically with concentration as abscissa and lysis time as ordinate, assays of TA and UK yielded linear curves with slope around —0.5. Inhibitor stock solutions in SBB were: EACA, 0.50 *M* (Sigma Chemical Company); PAMBA, 0.05 *M*; *trans-AMCHA*, 0.10 *M*. Inhibitor concentrations in the final mixture ranged from 10^{-9} to 0.096 *M*. Longest lysis times were 90 min.

Results and Discussion. The effects of the inhibitors on TA were uniform with the quantitative differences expected (Fig. 1). Inhibition of UK did not differ markedly from that of TA at low inhibitor concentrations (phase I). However, at medium concentrations of inhibitor, inhibition of UK decreased (phase II), turning in the case of EACA and AMCHA into a phase of enhancement of fibrinolysis. This was followed by a secondary rise in inhibition (phase III) at inhibitor concentrations much higher than those required to inhibit TA. These patterns of inhibition, for UK as well as for TA,

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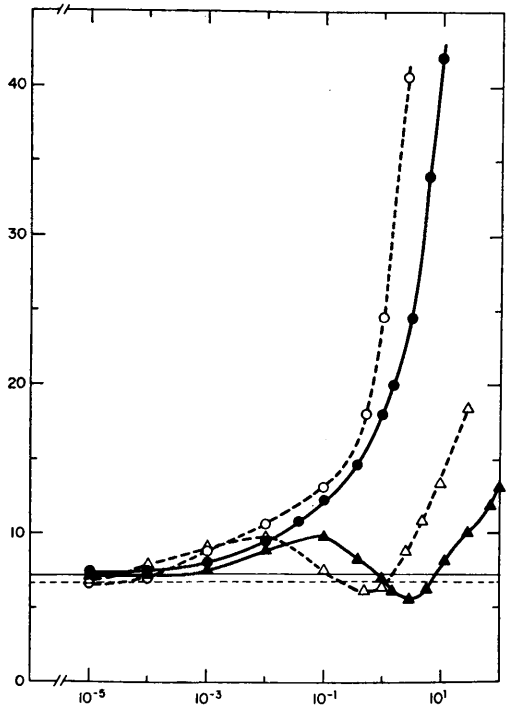

FIG. 1. Effects of EACA and AMCHA on lysis of fibrin by tissue plasminogen activator (TA) and urokinase (UK). (Abscissa) Inhibitor concentration in final mixture in m*M* (logarithmic); (ordinate); lysis time in minutes; activator concentration: 55 CTA units/ml of final mixture. (●), TA, EACA; (○), TA, AMCHA; (▲) UK, EACA; (△), UK, AMCHA; horizontal (— and - - -) lines indicate lysis times of controls without inhibitors.

remained the same within a range of activator activity corresponding to 2.5–80 CTA units/ml, and with activator preparations of different purity. Representative data are collected in Table I.

It is shown clearly that the inhibitors studied react with UK preparations in a manner different from the interaction with TA suggesting chemical differences between the two activators. It appears unlikely that impurities contaminating the preparations could produce differences as striking as those observed since activator preparations of different purity produced identical patterns of behavior. Other types of inhibitors (the bovine lung inhibitor, Kunitz pancreatic trypsin inhibitor, the urinary trypsin inhibitor, soy bean trypsin inhibitor, peanut trypsin inhibitor) did not exhibit such differences in behavior towards UK and TA.

TABLE I. Effects of EACA, AMCHA, and PAMBA on Tissue Plasminogen Activator (TA) and Urokinase (UK).[a]

	EACA	AMCHA	PAMBA
1. Lowest concentration inhibiting UK	3×10^{-3}	10^{-4}	3×10^{-4}
2. Lowest Concentration inhibiting TA	10^{-3}	10^{-4}	3×10^{-4}
3. Concentration at maximum inhibition of UK in phase I	10^{-1}	10^{-2}	10^{-2}
4. Maximum inhibition of UK in phase I (%)	47	58	57
5. Concentration at maximum enhancement of UK	3	0.5	1
6. Maximum enhancement of fibrinolysis (%)	59	26	0
7. Range of concentration producing enhancement	1–8	0.2–1	—
8. Concentration in second inhibitory phase producing same inhibition of UK as maximum inhibition in phase I	30	3	4.5
9. Ratio between nos. 8 and 3	300	300	450
10. Concentration producing 50% inhibition of UK in second inhibitory phase	32	2.5	4.0
11. Concentration producing 50% inhibition of TA	2.8×10^{-2}	2.8×10^{-3}	4.5×10^{-3}
12. Ratio between nos. 10 and 11	1140	890	890

[a] All inhibitor concentrations in millimol. The values are from single experiments with activator concentrations around 55 CTA units/ml of final reaction mixture producing a lysis time around 7 min in absence of inhibitor. Percentage inhibition and enhancement is expressed on the basis of concentrations calculated from reference curves of dilutions of TA and UK. Enhancement of fibrinolysis is recorded as the apparent increase in concentration of activator in percentage of activator present.

Alkjaersig *et al.* (14), using the fibrin plate method, found only minor differences in inhibitory effects of EACA on human UK and TA from pig heart. This discrepancy could be caused by differences in methods or in sources of TA. Recently, differences were observed in susceptibility of human UK and TA from pig heart to the inhibitory effect of plasma from certain patients (15). The enhancing effect of EACA on fibrinolysis reported by Alkjaersig *et al.* (14) was confirmed by several investigators (16). The peculiar biphasic inhibition of UK by EACA, AMCHA, and PAMBA is lacking with TA, and in additional experiments was absent also with plasmin, and was not produced by a different group of inhibitors. It is therefore evident that the effect is related to a specific interaction between UK and fibrin or plasminogen in the presence of the particular class of inhibitors studied. It cannot be excluded that impurities in the fibrinogen preparation could contribute to this interaction. It is puzzling how inhibition of UK by these inhibitors suddenly changes and becomes less by a factor of more than 100 and speculations about the mechanism involved would at this stage be purely conjectural. The described behavior is the most striking difference between UK and TA preparations so far reported.

Summary. Inhibition of porcine tissue plasminogen activator by ε-aminocaproic acid (EACA), trans-4-aminomethylcyclohexancarboxylic acid (trans-AMCHA) and *p*-aminomethylbenzoic acid (PAMBA) increased uniformly with increasing concentrations of inhibitor. In contrast, with human urokinase an early phase of inhibition at low inhibitor concentrations was followed by a phase of enhancement of fibrinolysis turning into a second phase of inhibition at inhibitor concentrations 300 times higher than required in the first phase. This biphasic inhibition of urokinase distinguishes it chemically from tissue plasminogen activator.

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